METHOD STATEMENT

Determinand:
Total and Faecal Coliforms organisms (including *E. Coli*)

Matrix:
Sample Type: Water samples from recreational and environmental sources and Treated sewage effluent.

Principle of Method:
Coliform organisms are members of a genus or species within the Enterobacteriaceae family that grow at 37°C and possess β-galactosidase. Coliform organisms are also oxidase negative. In the context of the method organisms that produce acid from lactose and form yellow colonies on the membrane filter are regarded as coliform organisms.
The MLGA contains lactose, an acidity indicator–phenol red and the chromagenic substrate BCIG (5-bromo-4-chloro-3-indolyl- β-glucoronide) either as a sodium or cyclohexylammonium salt. When hydrolysed BCIG indicates the presence of β-glucuronidase. Colonies that are β-glucuronidase positive are regarded as *E.coli*.
Faecal coliforms are defined as coliform bacteria that show thermo tolerance, producing yellow colonies coliforms at 44°C.
Volumes of sample, or appropriate aliquots of suitable dilutions, are filtered. The filters are incubated on absorbent pads soaked in Membrane Laurel Sulphate Broth (MLSB) for 4 hours at 30°C and 14 hours at 37°C (total coliforms) or for 4 hours at 30°C and 14 hours at 44°C (faecal coliforms).
The organisms produce characteristic yellow colonies on MLSB. Confirmation of presumptive isolates can be carried out by subculture to Tryptone Nutrient Agar (TNA) for 18-24 hours at 37°, followed by testing for the presence of β-galactosidase and the absence of oxidase.

Sampling and Sample Preparation:
Once taken, microbiological samples should be transferred immediately to dark storage conditions and kept at a temperature between 2 - 8°C for transport to the laboratory. If samples are not analysed immediately on receipt in the laboratory, they should be kept at a temperature between 2 - 8°C, in dark conditions until analysis commences.
Samples should be analysed as soon as practicable on the day of collection. In exceptional circumstances, if there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.
Where an exceedance has occurred, the customer should be informed or a statement reflecting this should be included with the report (except where the customer has been already made aware that this is occurring on a regular basis and requests this not to be applied).

Interferences
Samples with high turgidities may not be suitable for this method. The particulates may block the filter and limit the volume that can be examined. Accumulated deposits on the membrane may mask or inhibit the growth of the target organisms. High numbers of competing organisms may also mask or inhibit the growth of the target organisms.
Accumulated deposits on the filter membrane may also inhibit the growth of indicator organisms. For samples containing a lot of debris, the debris should be allowed to settle before filtration. More than one sample volume/dilution should also be filtered to enable easy reading of the final plates.
Some strains of Aeromonas, Staphylococcus and Bacillus may also produce yellow colonies. Confirmation procedures will determine their presence. Otherwise, this method is for presumptive Coliforms.

Performance of Method:

Range of Application:

N/A

Limit of Detection:

The usual limit of detection is 0 cfu/volume analysed. When a primary dilution of the sample is the first aliquot filtered the limit of detection will alter e.g. 1ml of a 10^{-1} dilution, the limit of detection would become <10cfu/ml

Uncertainty of measurement:

Not calculated

References:

The Microbiology of Recreational and Environmental Waters (2000).
Environment Agency – The Microbiology of Drinking Water (2002) – Part 4 – Methods for the Isolation and Enumeration of Coliform Bacteria and Escherichia Coli (including E. Coli 0157:H7).